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THE EFFECT OF ECO-ENZYME ON BIOMASS PRODUCTION OF *Lemna* sp. IN GIANT GOURAMI (*Osphronemus gouramy*) CULTURE SYSTEMS

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ABSTRACT

Giant gourami (*Osphronemus gouramy*) is one of Indonesia's most valuable freshwater fish species; however, its aquaculture development is constrained by slow growth and low survival rates. Improving water quality through the use of aquatic plants such as *Lemna* sp. and the application of eco-enzymes is essential. The development of *Lemna* sp. depends on nutrient availability, especially nitrogen (N), phosphate (P), and potassium (K), which can be enhanced by adding an eco-enzyme. This study aimed to evaluate the effect of different eco-enzyme concentrations on *Lemna* sp. biomass production, specific growth rate, and water quality parameters in giant gourami culture systems. The research was conducted using an experimental method with a Completely Randomized Design (CRD) consisting of five treatments and three replications: P0 (0 ml/L), P1 (1.5 ml/L), P2 (2.5 ml/L), P3 (3.5 ml/L), and P4 (4.5 ml/L) of eco-enzyme concentration. The results showed that eco-enzyme administration significantly influenced *Lemna* sp. biomass. The best results were observed in treatment P4 (4.5 ml/L), which produced the highest *Lemna* sp. biomass (132.87 g) and specific growth rate (4.47%). Optimal water quality was recorded in treatment P4, with temperature ranging from 26.1–29.8°C, pH 5.5–8.38, dissolved oxygen 3.10–6.42 mg/L, phosphate 0.3097–0.3872 mg/L, nitrate 0.5517–10.2414 mg/L, and CO_2 17–40 mg/L. These findings demonstrate that eco-enzyme application at 4.5 mL/L effectively enhances *Lemna* sp. productivity as a natural biofilter while simultaneously improving giant gourami growth performance and survival.

Keywords: Eco-enzyme, Duckweed, Integrated Aquaculture, Biomass Production

1. INTRODUCTION

The giant gourami (*Osphronemus gouramy*) is a freshwater fish of high economic value in Indonesia. This fish is widely cultivated due to high market demand, both for domestic consumption and export. Annual increases in public consumption support the high demand for giant gourami. The demand for giant gourami in Indonesia reached 176,113.78 tons in 2021. However, production figures are insufficient to meet this demand, as evidenced by KKP[1] data, which reached 149,170 tons.

This imbalance between demand and production is a particular concern, necessitating efforts to increase production through cultivation. According to research by Yusuf et al.[2], the main contributing factor is poor water quality in the culture medium, specifically high ammonia levels, which directly affect fish health and growth. Poor water quality, such as high ammonia levels, excessively high or low pH, and inappropriate temperatures, can cause stress in fish, slow growth, and increase mortality. Therefore, efforts to improve water quality are necessary through liming, fertilization, and the use of aquatic plants.

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One aquatic plant that can be used is *Lemna* sp., which helps maintain the balance of the aquatic environment. *Lemna* sp. can absorb inorganic nutrients, such as nitrogen and phosphate, from the water, thereby helping reduce levels of toxic compounds, such as ammonia[3]. The biomass growth of *Lemna* sp. can serve as an indicator of good water quality, as this plant is susceptible to changes in the aquatic environment. Therefore, the success of giant gourami cultivation can also be supported by the presence and increase in biomass of *Lemna* sp. in the cultivation medium. *Lemna* sp. growth is influenced by the availability of nutrients, especially nitrogen (N), phosphate (P), and potassium (K). These nutrients must be adequate for optimal growth. One way to increase water nutrient levels is by adding ecoenzymes.

Some of the nutrients required by plants are found in ecoenzymes, namely nitrogen (N), phosphorus (P), and potassium (K)[4]. Several previous studies have demonstrated the benefits of ecoenzymes in fish farming. The application of ecoenzymes using simple aquaponics technology at a dose of 15 ml/L increased the specific growth rate of fish by 4.19%. However, research on the use of ecoenzymes on *Lemna* sp. biomass in giant gourami cultivation has not yet been conducted, requiring further study.

This study aimed to: (1) evaluate the effect of different eco-enzyme concentrations (0, 1.5, 2.5, 3.5, and 4.5 mL/L) on *Lemna* sp. biomass production and specific growth rate in giant gourami culture systems, (2) assess the impact of eco-enzyme supplementation on water quality parameters including temperature, pH, dissolved oxygen, phosphate, nitrate, and carbon dioxide concentrations, and (3) determine the optimal eco-enzyme concentration for maximizing *Lemna* sp. productivity while maintaining acceptable water quality conditions for fish culture. The findings are expected to provide practical guidelines for implementing eco-enzyme supplementation in integrated *Lemna* sp.-fish aquaculture systems, contributing to more sustainable and productive aquaculture practices.

2. RESEARCH METHOD

Time and Place

The study was conducted 30 days from March to April 2025 at the Aquaculture Environmental Quality Laboratory, Faculty of Fisheries and Marine, Universitas Riau. Nitrate, phosphate, and CO_2 measurements were conducted at the Marine Science Laboratory, Faculty of Fisheries and Marine, Universitas Riau.

Method

This study employed an experimental method using a completely randomized design (CRD) with five treatment levels and three replications. However, the application of eco-enzyme for *Lemna* sp. cultivation in giant gourami rearing systems required further preliminary testing to establish the optimal dosage. The eco-enzyme concentrations used in this study were determined based on initial trials and consisted of the following treatments:

P0: control (0 ml/L eco-enzym)
P1: 1.5 mL/L eco-enzyme concentration
P2: 2.5 mL/L eco-enzyme concentration
P3: 3.5 mL/L eco-enzyme concentration
P4: 4.5 mL/L eco-enzyme concentration

Procedures

Eco-enzyme Production

Eco-enzyme was produced following the protocol described by Purba et al.[5]. The production process began with collecting raw materials, including vegetable and fruit peels, sugar (or molasses), and water. The organic waste materials were thoroughly washed with running water to remove dirt and contaminants, then chopped into small pieces to facilitate fermentation. Sugar was dissolved in water by continuous stirring until completely homogenized. The ingredients were mixed in a 1:3:10 ratio (sugar: organic waste: water). After mixing, the solution was transferred into a fermentation container and loosely covered

to allow the release of fermentation gases. The fermentation process was carried out for three months at ambient temperature before the eco-enzyme was harvested and applied in the experiment.

Container Preparation

The experimental units consisted of black plastic buckets measuring 60 cm in diameter and 45 cm in height, with a maximum capacity of approximately 100 L. Prior to use, all containers were thoroughly cleaned by scrubbing the interior walls with soap to remove any residues, then rinsed with clean water. Each container was then filled with 40 L of water obtained from a storage tank with an initial pH of 5.0–5.5. One aeration unit was installed in each container to maintain adequate dissolved oxygen levels throughout the study period.

***Lemna* sp. Cultivation**

Following an adaptation period, *Lemna* sp. were stocked in the rearing containers at a density of 47 g/m², as per Nisa[6]. The *Lemna* sp. were distributed evenly across the water surface to provide partial shading without impeding gas exchange or light penetration. Throughout the cultivation period, *Lemna* sp. were monitored daily for growth and health status. *Lemna* sp. biomass was sampled every two days to assess growth rate and biomass accumulation. Biomass measurements were conducted by harvesting all *Lemna* sp. from each container, blotting to remove excess water, and weighing the fresh biomass on an analytical balance (precision ±0.01 g). The biomass values were recorded for subsequent analysis.

Fish Rearing

The experimental fish consisted of giant gourami fingerlings measuring 5–6 cm in total length. Before stocking, fingerlings were carefully selected for uniform size, active swimming behavior, and the absence of physical defects or injuries. The stocking density was determined according to Jernihtayanti et al.[7] at 1 fish per 4 L of water. With a water volume of 40 L per container, 10 fingerlings were stocked in each experimental unit. The rearing period lasted 30 days. Fish were fed three times daily (08:00, 12:00, and 16:00 WIB) with commercial feed (Prima Feed-800) at a feeding rate of 3% of total fish biomass per day. The feeding rate was adjusted following each sampling event based on the updated total biomass.

Water Quality Monitoring

Water quality parameters were monitored throughout the study according to standard protocols. Temperature and pH were measured three times daily (morning, afternoon, evening) using a digital pH/temperature meter (Hanna HI98128). Dissolved oxygen (DO) was measured using a portable DO meter (Lutron DO-5510) at the beginning, middle (day 15), and end (day 30) of the study. Water samples for chemical analyses (phosphate, nitrate, and CO_2) were collected at the same time points in clean polyethylene bottles and immediately transported to the laboratory for analysis.

Phosphate concentration was determined by the ascorbic acid method using a spectrophotometer (Genesys 20) at 880 nm wavelength following standard methods. Nitrate concentration was analyzed using the cadmium reduction method followed by spectrophotometric determination at 543 nm. Carbon dioxide concentration was determined by titration with sodium hydroxide using phenolphthalein as an indicator[8].

***Lemna* sp. Biomass Increase**

Lemna sp. biomass increase was measured every two days by weighing all *Lemna* sp. in the research container. According to Sogbesan et al.[9], the formula for calculating *Lemna* sp. biomass increase is as follows:

$$Wn = Wt - Wo$$

Note:

Wn : Absolute weight increase (g).
Wt : *Lemna* sp. biomass at the end of the study (g).

Wo : *Lemna* sp. biomass at the beginning of the study (g).

Specific Growth Rate (SGR) of *Lemna* sp.

The specific growth rate was calculated using the formula from Effendie[10].

$$\alpha = \frac{LnWt - LnWo}{t} \times 100\%$$

Where:

α : Specific growth rate (g/day %)
Wo : Average weight of *Lemna* sp. at the beginning of the study (g)
Wt : Average weight of *Lemna* sp. at the end of the study (g)
t : Length of maintenance (days).

3. RESULT AND DISCUSSION

Eco-Enzyme Chemical Composition

The material used as a treatment for *Lemna* sp. in the rearing media of Giant gourami was eco-enzyme-fermented for 3 months. The results of the chemical analysis of the eco-enzyme fermented for 3 months are presented in Table 1.

Table 1. Chemical Analisys of Eco-Enzyme

No	Component	Concentration (%)
1	Organic Carbon (C)	0,30
2	Nitrogen (N)	0,24
3	Phosphorus (P)	0,11
4	Potassium (K)	0,31

Based on the chemical analysis results presented in Table 1, the eco-enzyme contained 0.30% organic carbon (C), 0.24% nitrogen (N), 0.11% phosphorus (P), and 0.31% potassium (K). The organic carbon content serves as a food source for microorganisms; thus, by stimulating microbial activity, it can enhance the decomposition process of organic matter in nutrient-rich aquatic environments. According to Hardjowigeno[11], organic carbon content is a crucial indicator of substrate fertility and nutrient cycling capacity. The organic carbon level in the eco-enzyme (0.30%) is considered low for optimal plant growth support, as Sari & Yusmah[12] classified organic carbon content below 1% as very low. However, this lower carbon content may reduce the risk of oxygen depletion in the water column, which is beneficial in fish rearing systems[8].

Nitrogen (N) is a macronutrient used by plants as a structural component of proteins. Wahyudin et al.[13] emphasized that nitrogen plays a critical role in protein synthesis and is fundamental for plant growth and development. The chemical analysis revealed a nitrogen content of 0.24% in the eco-enzyme, which falls within the optimal range for *Lemna* sp. growth. Tekogul et al.[14] reported that the optimal nitrogen requirement for *Lemna* sp. ranges from 0.042% to 0.63%, indicating that the nitrogen content in the eco-enzyme used in this study is adequate to support robust *Lemna* sp. growth.

Phosphorus (P) is a macronutrient involved in energy metabolism, particularly ATP formation, nucleic acid synthesis, and new tissue growth[15]. The phosphorus content in the eco-enzyme was 0.11%, which is relatively low for supporting maximum *Lemna* sp. growth. Chapman & Boucher[16] noted that the optimal phosphorus requirement for *Lemna* sp. is approximately 0.35%, suggesting that the phosphorus level in the eco-enzyme (0.11%) is below the optimal threshold, indicating that the value of 0.11% falls within the adequate range but is still considered low for promoting maximum growth.

Potassium (K) functions as an enzyme activator, regulates osmotic balance, and maintains cell turgor pressure, all of which are essential for plant structural integrity and stress tolerance[17]. The potassium content of the eco-enzyme was 0.31%, which is categorized as adequate for plant growth.

The nutrient composition of the eco-enzyme demonstrates its potential as a sustainable and cost-effective fertilizer for enhancing *Lemna* sp. productivity in integrated aquaculture systems. The balanced macronutrient content, particularly nitrogen and potassium, provides essential nutrients for plant growth while minimizing the risk of water quality degradation

associated with excessive organic loading[18]. However, the relatively low phosphorus content suggests that supplementary phosphorus sources may be beneficial for maximizing *Lemna* sp. biomass production in systems with high nutrient demands.

Water Quality

In this study, the application of eco-enzyme to *Lemna* sp. in the rearing media of giant gourami over 30 days involved monitoring several critical water quality parameters, including temperature, pH, dissolved oxygen (DO), phosphate (PO_4^{3-}), nitrate (NO_3^-), and carbon dioxide (CO_2). The results of water quality measurements throughout the experiment are presented in Table 2.

The optimal water quality conditions for enhancing *Lemna* sp. biomass in giant gourami rearing systems were observed in treatment P4. In this treatment, the measured parameters included temperature of 26.1–29.8°C, pH of 5.5–8.38, DO of 3.10–6.42 mg/L, phosphate of 0.3097–0.3872 mg/L, nitrate of 0.5517–10.2414 mg/L, and CO_2 of 17–40 mg/L. The water quality parameters in P4 supported the highest *Lemna* sp. These findings are consistent with Kristiana et al.[19], who reported that ideal water quality for *Lemna* sp. growth typically includes a pH of 6–8 and a temperature range of 25–30°C. Maintaining stable water quality parameters is crucial for optimizing both plant productivity and fish growth in integrated aquaculture systems[8].

Table 2. Water quality

Parameter	Observation Results				
	P0	P1	P2	P3	P4
Temperature (°C)	26.4-29.53	26.63-29.8	26.5-29.8	25.9-30.6	26.1-29.8
pH	5.5-8.11	5.5-8.30	5.5-8.27	5.5-8.32	5.5-8.38
DO (mg/L)	6.23-6.70	5.77-6.54	4.1-6.8	3.47-7.01	3.10-6.42
Phosphate (mg/L)	0.1637-0.7633	0.1726-1.0597	0.3827-1.1571	0.1881-0.4004	0.3097-0.3872
Nitrate (mg/L)	0.0543-4.2658	0.2627-7.5394	0.4308-8.4125	0.2364-8.9412	0.5517-10.2414
CO_2 (mg/L)	5-8	7-18	10-32	12-28	17-40

Temperature

Based on Table 2, the temperature in treatment P4 ranged from 26.1 to 29.8°C, which was similar to the ranges observed in the other treatments. These values fall within the optimal range for the growth of both *Lemna* sp. According to Kristiana et al.[19], the optimal temperature range for *Lemna* sp. growth is 25–30°C, as temperatures within this range maximize photosynthetic efficiency and biomass production (Figure 1).

The daily temperature patterns among treatments show a similar trend. Temperature increased from the beginning to the middle of the study period, then fluctuated slightly, remaining within the 27–30°C range. There were no significant differences among treatments, indicating that all treatments experienced relatively uniform temperature conditions.

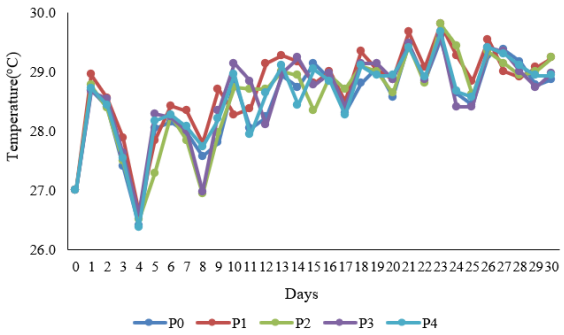

Figure 1. Temperature measurement

The temperature profile shown in the graph indicates that the water temperature throughout the experiment remained within the optimal range for *Lemna* sp. growth. Suboptimal temperatures for *Lemna sp.* can prolong the adaptation phase, resulting in slower growth. This is supported by Mateo-Elizalde et al.[20], who stated that under extreme weather conditions, such as low temperatures, *Lemna* sp. enters a dormancy phase in which it produces turions with

genetic characteristics similar to the parent plant, sinking them to the bottom of the water body until environmental conditions become favorable for growth.

The fluctuations observed in the graph remained within normal limits (±2–3°C) and were therefore unlikely to cause environmental stress. Thus, it can be concluded that water temperature across all treatments was stable and uniform, and did not act as a determining factor in the outcomes of this study.

pH

Based on Table 1, observations during the 30-day culture period showed that the media pH in treatments with eco-enzyme addition ranged from 5.5 to 8.38. These values fall within the acceptable range for supporting the growth of *Lemna sp.* biomass. Both excessively low and excessively high pH levels may pose risks of stress to the fish.

In this study, although pH values across P0-P4 showed some variation, the eco-enzyme treatments appeared capable of maintaining pH stability, particularly in P4, which ranged from 5.5 to 8.38. The pH measurement graph throughout the experiment is presented in Figure 2.

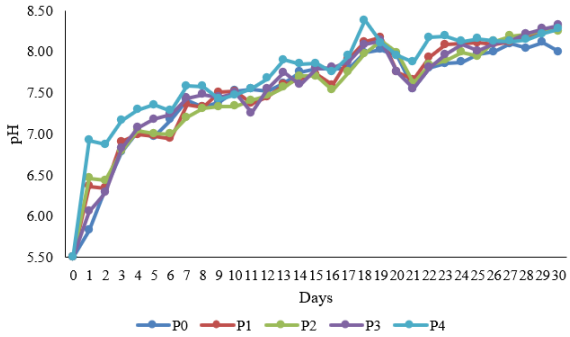

Figure 2. pH Measurement

This stability is presumed to be related to microbial activity within the eco-enzyme, which contributes to the decomposition and mineralization of organic matter. Furthermore, the presence of *Lemna sp.* in the medium also influences pH. *Lemna sp.* is an aquatic plant that actively performs photosynthesis during the daytime by utilizing dissolved CO_2. According to Suhartawan et al.[21], when CO_2 dissolves in water, it reacts to form carbonic acid (H_2CO_3), which can dissociate into hydrogen ions (H^+) and bicarbonate ions (HCO_3^-). Thus, *Lemna sp.* functions not only as a nutrient absorber (e.g., nitrate and phosphate) but also as a biological factor that stabilizes water pH.

Dissolved Oxygen (DO)

Based on Table 2, dissolved oxygen (DO) concentrations observed during the 30-day culture period ranged from 3.10 to 6.70 mg/L across treatments with eco-enzyme addition. These values generally remain within the acceptable range to support *Lemna* sp. biomass production. According to Sitanggang & Sarwono[22], the optimal DO concentration for giant gourami is 4-6 mg/L. The DO measurement graph throughout the study is presented in Figure 3.

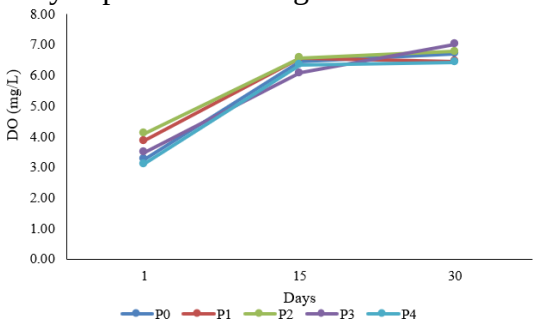

Figure 3. Dissolved Oxygen (DO)

Based on Figure 3, dissolved oxygen levels in the rearing media increased over the 30 days across all treatments. On Day 1, the DO value in P4 (3.10 mg/L) was lower than in P1 (6.23 mg/L) and P2 (5.77 mg/L), which had higher concentrations. The lower pH levels influenced this condition. This is supported by Suhartawan et al.[21], who stated that when pH becomes too low, photosynthesis can be inhibited, reducing oxygen production and affecting overall water quality.

By Day 15, the graph showed a significant increase in DO across nearly all treatments, with values ranging from 6.07 to 6.60 mg/L. By Day 30, DO levels continued to increase, reaching 6.42–7.01 mg/L, with P3 showing the highest value (7.01 mg/L). Overall, the graph indicates that after an

initial decrease, DO levels in all treatments eventually stabilized within a range favorable for aquatic organisms.

This condition also suggests that *Lemna* sp.'s photosynthetic activity contributed to oxygen production. This is supported by Amelia et al.[23], who stated that photosynthesis converts inorganic compounds (CO_2 and H_2O) into organic compounds (carbohydrates) and O_2 using sunlight.

Phosphate

Based on Table 1, the phosphate concentrations measured during the study on the application of eco-enzyme to *Lemna* sp biomass in the rearing of Giant gourami ranged from 0.1637 to 1.1571 mg/L. The phosphate concentration in P4 ranged from 0.3097 to 0.3872 mg/L. The phosphate concentration observed in this study remains insufficient for optimal *Lemna* sp. growth. Ramadhan et al.[24] also stated that higher availability of phosphate and nitrate in the water can enhance the growth of *Lemna sp.* The effect of eco-enzyme dosage on phosphate concentration is shown in Figure 4.

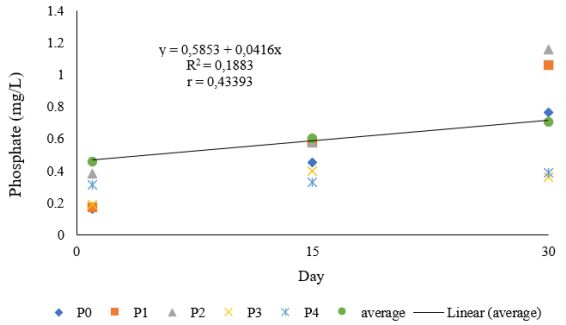

Figure 4. Phosphate

Figure 4 illustrates the relationship between eco-enzyme concentration and phosphate levels in the culture medium, represented by the linear regression equation $y = 0.583 – 0.0416x$, where y represents phosphate concentration (mg/L) and x represents eco-enzyme dosage (ml/L). The coefficient of determination (R^2) was 0.1883, and the Pearson correlation coefficient (r) was 0.4339. The positive value of r = 0.4339 indicates a weak-to-moderate positive correlation between increasing eco-enzyme dosage and phosphate concentration in the water column. According to Suprapto[25], correlation coefficients are categorized as weak ($0.20 < r \leq 0.40$), moderate ($0.40 < r \leq 0.70$), or strong ($r > 0.70$). With r = 0.4339, the relationship between eco-enzyme concentration and phosphate levels falls at the lower boundary of the moderate correlation category.

However, it is important to note that the correlation coefficient alone does not account for the proportion of variance explained by the independent variable. The R^2 value provides this information, indicating the extent to which variation in eco-enzyme concentration explains changes in phosphate levels[26]. The R^2 value of 0.1883 indicates that only 18.83% of the variation in phosphate concentration can be explained by eco-enzyme dosage. In comparison, the remaining 81.17% is influenced by other factors not captured by the regression model. According to Maharadja et al.[27], an R^2 value of 0.00–0.199 indicates a weak relationship between the independent and dependent variables, suggesting limited predictive power for the model.

Nitrate

Based on Table 1, the nitrate values measured during the application of eco-enzyme on *Lemna* sp. biomass in Based on Table 2, nitrate (NO_3^-) concentrations measured during the application of eco-enzyme to enhance *Lemna* sp. biomass in giant gourami culture systems ranged from 0.0543 to 10.2414 mg/L across all treatments. The nitrate concentration in treatment P4 ranged from 0.5517 to 10.2414 mg/L, the highest among all treatments and the most significant temporal variation throughout the study period. These concentrations fall within or near the optimal range for *Lemna* sp. growth. The effect or correlation between eco-enzyme dosage and nitrate levels is shown in Figure 5

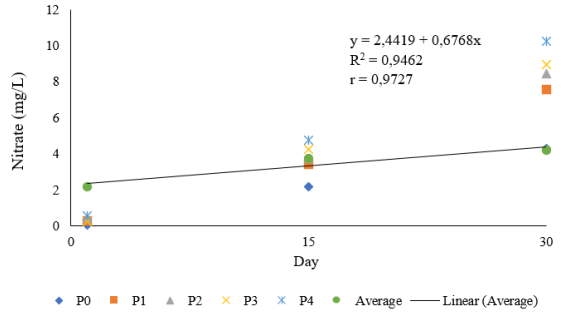

Figure 5. Nitrate Regression Analysis

Based on Figure 5, nitrate concentrations exhibited distinct temporal patterns that varied among treatments, with a general trend toward increasing concentrations over time across all treatments. The control treatment (P0) maintained relatively low nitrate levels throughout the study (0.0543–4.2658 mg/L), reflecting limited nitrogen input in the absence of eco-enzyme supplementation. In contrast, treatments with eco-enzyme addition (P1–P4) showed progressively higher nitrate concentrations, with the increase most pronounced at higher eco-enzyme dosages.

The R^2 value in Figure 10 measures the extent to which eco-enzyme dosage explains the variation in nitrate concentration. An R^2 value of 0.9462 indicates that 94.62% of the increase in nitrate concentration is explained by eco-enzyme dosage, while other factors influence the remaining 5.38%. According to Maharadja et al.[27], an R^2 value of 0.80–1.000 indicates a very strong relationship, suggesting that eco-enzyme application is a major factor influencing increases in nitrate levels.

The increase in nitrate concentration in treatment P4 is likely due to the eco-enzyme's role in enhancing the efficiency of nitrification in the water. Eco-enzymes contain active organic compounds and fermentation-derived enzymes that may increase the activity of nitrifying microorganisms. This is supported by Tong & Liu[28], who reported that eco-enzymes increase total nitrogen and organic matter levels by activating enzymes such as trypsin and amylase, as well as by producing organic acids. Although these enzymes do not react directly with the water, they enhance the performance of microorganisms in aquatic environments. Similarly, Susilowati et al.[29] stated that enzymes contained in eco-enzymes strengthen microorganisms involved in organic matter decomposition, stimulate plant growth, and act as agents for controlling plant pests and diseases.

CO_2

Based on Table 1, the CO_2 values measured during the study on the application of eco-enzyme to *Lemna sp.* biomass in giant gourami culture ranged from 5 to 40 mg/L. The CO_2 values in treatment P4 ranged from 17 to 40 mg/L (Figure 6).

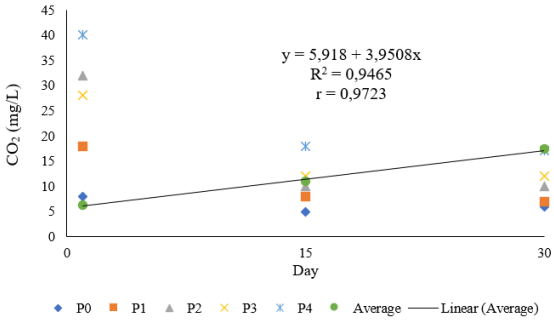

Figure 6. CO_2 Regression Analysis

Figure 6 illustrates the relationship between eco-enzyme concentration and CO_2 levels in the culture medium, represented by the linear regression equation $y = 5.918 + 3.9508x$, where y represents CO_2 concentration (mg/L) and x represents eco-enzyme dosage (mL/L). The coefficient of determination (R^2) was 0.9465, and the Pearson correlation coefficient (r) was 0.9723. The exceptionally high positive correlation coefficient (r = 0.9723) indicates a strong, positive linear relationship between increasing eco-enzyme dosage and increasing CO_2 concentrations in the culture system. According to Suprapto[25], correlation coefficients are considered very strong when $0.90 < r \leq 1.00$, indicating that the variables are highly associated and exhibit a highly predictable relationship.

The R^2 value of 0.9465 provides crucial information about the model's explanatory power, indicating that 94.65%

of the variation in CO_2 concentration is explained by eco-enzyme dosage. In comparison, 5.35% is explained by other factors not captured by the regression model[26]. According to Maharadja et al.[27], an R^2 value of 0.80–1.00 indicates a very strong relationship between variables, demonstrating excellent model fit and predictive capability. This remarkably high R^2 value confirms that eco-enzyme application is the primary factor affecting CO_2 dynamics in the experimental system, with minimal influence from confounding variables.

CO_2 is beneficial for *Lemna* sp. because it is used in photosynthesis to support growth and biomass production. According to Suhartawan et al.[21], dissolved carbon dioxide is the primary carbon source for photosynthesis in aquatic plants and algae. Boyd[30] stated that optimal CO_2 levels for cultured fish are <5 mg/L, while Schmidt and Goldbach[31] reported that aquatic plants such as *Lemna* sp. can utilize dissolved CO_2 up to concentrations <50 mg/L for photosynthesis. In this study, the highest CO_2 concentration was recorded in P4 (17–40 mg/L). This indicates that part of the CO_2 concentration in P4 is still usable by *Lemna* sp. for photosynthesis, although it exceeds the optimal level for giant gourami.

Biomass and Specific Growth Rate (SGR) of *Lemna* sp

The initial biomass of *Lemna* sp. was 47 g per experimental container, corresponding to a density of 47 g/m² based on the container surface area. The observed increase in biomass and the specific growth rate (SGR) of *Lemna* sp. during the 30-day experiment with different eco-enzyme concentrations in each treatment are presented in Table 3.

Table 3. Average Biomass Growth and Specific Growth Rate (SGR) of *Lemna sp.*

Treatment	Biomass (g)	Specific Growth Rate (%)
P0 (Kontrol)	99.83 ± 0.67^{a}	3.79 ± 0.15^{a}
P1 (1.5 ml/L)	104.39 ± 1.34^{b}	3.89 ± 0.03^{b}
P2 (2.5 ml/L)	118.39 ± 0.55^{c}	4.19 ± 0.01^{c}
P3 (3.5 ml/L)	120.77 ± 0.90^{c}	4.24 ± 0.02^{c}
P4 (4.5 ml/L)	132.87 ± 2.74^{d}	4.47 ± 0.05^{d}

Note: Different superscript letters in the same column indicate significant differences ($P < 0.05$)

Based on Table 3, eco-enzyme application significantly influenced both final biomass and the specific growth rate of *Lemna* sp. ($p < 0.05$), with effects that were dose-dependent across the tested concentration range. The control treatment (P0) without eco-enzyme supplementation produced a final biomass of 99.83 ± 0.67 g and an SGR of 3.79 ± 0.15% day^{-1}, representing a 112.4% increase from the initial stocking biomass of 47 g.

The highest biomass increase was observed in P4, reaching 132.87 g. This increase is presumed to be due to the higher eco-enzyme dosage, which met the nutrient requirements for *Lemna sp.* growth. Eco-enzyme contains N, P, and K, which are essential for *Lemna* sp. development. This is consistent with Salsabila & Winarsih[4], who state that the eco-enzyme solution contains nutrient elements such as N, P, and K. The presence of these nutrients can increase the availability of nitrogen and phosphorus in the medium. According to Natalina et al.32, nutrient content, such as nitrate and phosphate, originates from the decomposition of organic fertilizers by beneficial decomposer bacteria, thereby improving fertilizer performance.

The lowest biomass increase in P0 occurred because no eco-enzyme was added, meaning that the only nutrient sources available for *Lemna* sp. were fish feces, decaying plant matter, and feed residues, which are insufficient for optimal growth. This aligns with Stathopoulou et al.[32], who

stated that metabolic waste and uneaten feed can act as fertilizer for plants. Additionally, the lack of decomposer bacterial activity reduces the availability of key nutrients, including nitrate. Wikaningrum & Dabo[34] reported that the application of eco-enzymes increases microbial activity, thereby promoting the nitrification of ammonia to nitrate in aquatic systems.

Analysis of Variance (ANOVA) showed that different eco-enzyme dosages had a significant effect on the biomass growth of *Lemna* sp. ($P < 0.05$). In this study, the highest biomass increase was obtained in P4 at 132.87 g. These results were lower than those reported by Ramadhan et al.[24], who found that the application of liquid organic fertilizer (LOF) from catfish produced a biomass of 767.47 g/m². This difference is attributed to the lower organic C, N, P, and K content in the eco-enzyme (Table 5) compared to the LOF, which contained 3.17 g organic C, 0.35 g organic N, 0.19 g organic P, and 0.43 g organic K. Higher levels of N, P, and K in LOF ensure sufficient nutrient supply for *Lemna* sp. This is supported by Zenir et al.35, who stated that *Lemna* sp. growth is highly dependent on nutrient availability in the culture medium. As shown in Table 5, different eco-enzyme dosages also significantly affected the Specific Growth Rate (SGR) of *Lemna* sp. ($P < 0.05$). The highest SGR was found in P4 (4.5 mL/L), reaching 4.47%/day, while the lowest SGR was recorded in P0 (3.79%/day).

The highest SGR in *Lemna* sp. was observed in P4, at 4.47%/day. This is presumably due to the increasing eco-enzyme dosage, which accelerated nutrient availability and resulted in faster SGR compared to treatments without eco-enzyme. Zenir et al.[35] explained that *Lemna* sp. growth is highly dependent on nutrient levels in the culture medium. Under nutrient-deficient conditions, the plant can only grow to a limited extent because nutrients are insufficient to support optimal metabolism and tissue synthesis. ANOVA results showed that different eco-enzyme dosages had a significant effect on the SGR of *Lemna* sp. ($P < 0.05$

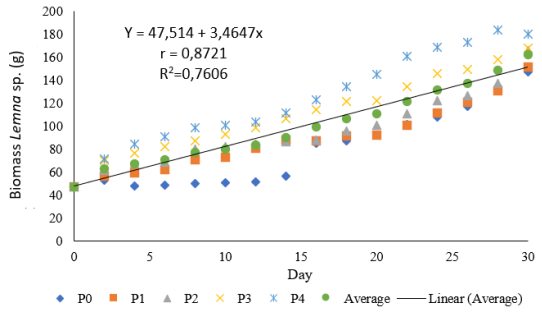

Figure 7. Regression graph of the effect of eco-enzyme dosage on the growth of *Lemna* sp

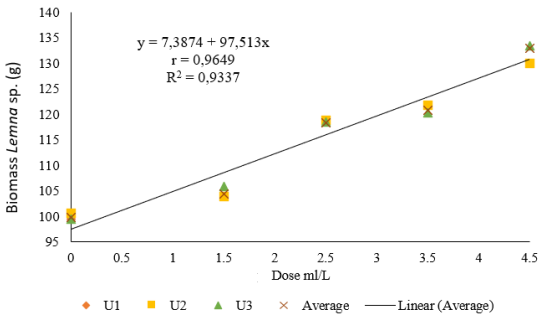

Figure 8. The effect of rearing duration on the increase in *Lemna* sp. biomass

Figure 7 shows the relationship between eco-enzyme dosage and *Lemna* sp. biomass, represented by the linear regression equation $y = 7.3894 + 97.513x$, with a coefficient of determination (R^2) of 0.9337 and a correlation coefficient (r) of 0.9649. The value $r = 0.9649$ indicates a very strong, positive correlation between increasing eco-enzyme dosage and increased *Lemna sp.* biomass. According to Suprapto[25], a correlation is categorized as very strong when $0.90 < r \leq 1.00$.

The R^2 value in Figure 8 indicates the proportion of variation in *Lemna* sp. biomass explained by eco-enzyme dosage. An R^2 of 0.9337 indicates that 93.37% of the variation in biomass growth is explained by eco-enzyme dosage, with the remaining 6.63% attributed to other factors. According to Maharadja et al.[27], an R^2 range of 0.80–1.000 indicates a very strong relationship;

therefore, it can be concluded that eco-enzyme dosage is a significant factor influencing the growth of *Lemna* sp.

Figure 8 shows the relationship between rearing duration and *Lemna* sp. biomass, represented by the linear regression equation y = 47.514 + 3.4647x, with a coefficient of determination (R^2) of 0.7606 and a correlation coefficient (r) of 0.8721. The value r = 0.8721 indicates a strong, positive correlation between increasing rearing duration and increased *Lemna* sp. biomass. According to Suprapto[25], this value falls within the strong correlation range, defined as $0.70 < r \leq 0.90$.

The R^2 value in Figure 13 indicates how much of the variation in *Lemna* sp. biomass is explained by the length of the rearing period. An R^2 of 0.7606 means that 76.06% of the variation in biomass growth can be explained by rearing duration, while other factors account for the remaining 23.94%. According to Maharadja et al.[27], an R^2 range of 0.60–0.799 indicates a strong relationship. Thus, it can be concluded that rearing duration plays a strong role in the growth of *Lemna* sp. biomass, although its influence is lower than that of eco-enzyme dosage.

The regression analysis indicates that eco-enzyme dosage contributes substantially more to the increase in *Lemna* sp. biomass compared to rearing duration, as evidenced by the significant differences in R^2 and r values between the two graphs.

4. CONCLUSION

This study demonstrates that eco-enzyme application significantly enhances *Lemna* sp. biomass production in integrated giant gourami culture systems, with effects being dose-dependent across the concentration range tested (0–4.5 mL/L). The optimal treatment was achieved with eco-enzyme supplementation at 4.5 mL/L (P4), which produced the highest *Lemna* sp. final biomass of 132.87 ± 2.74 g (representing a 182.7% increase from initial stocking biomass) and the highest specific growth rate of 4.47 ± 0.05% day^{-1}, observed in treatment P2 (2.5 mL/L), suggesting that a trade-off exists between maximizing growth performance and optimizing survival at the highest eco-enzyme concentrations. Water quality parameters in treatment P4 remained generally within acceptable ranges for both *Lemna* sp. cultivation and giant gourami culture. However, some parameters approached threshold values: temperature 26.1–29.8°C, pH 5.5–8.38, dissolved oxygen 3.10–6.42 mg/L, phosphate 0.3097–0.3872 mg/L, nitrate 0.5517–10.2414 mg/L, and carbon dioxide 17–40 mg/L.

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